

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (currently amended) A biofuel cell for generating electricity comprising:

~~— a fuel fluid;~~

~~— an electron mediator;~~

~~— a cathode capable of reducing an oxidant in the presence of electrons to form~~  
5 ~~water; and~~

~~— a bioanode which comprises comprising~~

(a) an electron conductor;

(b) at least one enzyme capable of reacting with an oxidized form of an the  
electron mediator and a the fuel fluid to produce an oxidized form of the fuel fluid and a  
10 reduced form of the electron mediator;

(c) an enzyme immobilization material capable of immobilizing and stabilizing the  
enzyme, the material being permeable to the fuel fluid and the electron mediator; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the  
electrocatalyst being capable of reacting with the reduced form of the electron mediator  
15 to produce an oxidized form of the electron mediator and a reduced form of the  
electrocatalyst, the reduced form of the electrocatalyst being capable of releasing  
electrons to the electron conductor.

2. (currently amended) A biofuel cell for generating electricity comprising:

~~— a fuel fluid;~~

~~— a cathode capable of reducing an oxidant in the presence of electrons to form~~  
water; and

5 ~~— a bioanode which comprises comprising~~

(a) an electron conductor;

(b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and ~~a~~ the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

10 (c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator  
15 to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor.

3. (currently amended) The bioanode ~~biofuel cell~~ of claim 1 wherein the enzyme immobilization material comprises a micellar or inverted micellar structure, the material being permeable to the fuel fluid and the electron mediator.

4. (currently amended) The bioanode ~~biofuel cell~~ of claim 2 wherein the enzyme immobilization material comprises a micellar or inverted micellar structure, the material being permeable to the fuel fluid.

5. (currently amended) ~~A biofuel cell for generating electricity comprising:~~

~~— a fuel fluid;~~

~~— an electron mediator;~~

~~— a cathode capable of reducing an oxidant in the presence of electrons to form  
5 water; and~~

~~— a bioanode for oxidizing the fuel fluid to generate electricity, the bioanode comprising~~

(a) an electron conductor;

10 (b) at least one enzyme capable of reacting with an oxidized form of an the electron mediator and a the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor; and

(c) an enzyme immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid and the electron mediator.

6. (currently amended) ~~A biofuel cell for generating electricity comprising:~~

~~—— a fuel fluid;~~

~~—— a cathode capable of reducing an oxidant in the presence of electrons to form water; and~~

5 ~~—— a bioanode for oxidizing the fuel fluid to generate electricity, the bioanode comprising~~

(a) an electron conductor;

10 (b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and a the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid.

7. (currently amended) The bioanode ~~biofuel cell~~ of claim 5 wherein the enzyme immobilization material comprises a micellar or inverted micellar structure, the material being permeable to the fuel fluid and the electron mediator.

8. (currently amended) The bioanode biofuel cell of claim 6 wherein the enzyme immobilization material comprises a micellar or inverted micellar structure, the material being permeable to the fuel fluid.

9. (currently amended) The bioanode biofuel cell of claim 5 wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-PTFE copolymer, the material being permeable to the fuel fluid and the electron mediator.

10. (currently amended) The bioanode biofuel cell of claim 6 wherein the enzyme immobilization material comprises an alkylammonium salt extracted perfluoro sulfonic acid-PTFE copolymer, the material being permeable to the fuel fluid.

11. (currently amended) The bioanode biofuel cell of claim 1 wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-PTFE copolymer, the material being permeable to the fuel fluid and the electron mediator.

12. (currently amended) The bioanode biofuel cell of claim 6 2 wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-PTFE copolymer, the material being permeable to the fuel fluid.

13. (currently amended) The bioanode biofuel cell of claim 6 2 wherein the electron conductor comprises a carbon-based material, a metallic conductor, a semiconductor, a metal oxide or a modified conductor.

14. (currently amended) The bioanode biofuel cell of claim 6 2 wherein the electron conductor comprises carbon cloth, carbon paper, carbon screen printed electrodes, carbon black, carbon powder, carbon fiber, single-walled carbon nanotubes, double-walled carbon nanotubes, multi-walled carbon nanotubes, carbon nanotube

5 arrays, diamond-coated conductors, glass carbon, mesoporous carbon, graphite,  
uncompressed graphite worms, delaminated purified flake graphite, high performance  
graphite, highly ordered pyrolytic graphite, pyrolytic graphite, polycrystalline graphite,  
gold, platinum, iron, nickel, copper, silver, stainless steel, mercury, tungsten,  
nanoparticles made of cobalt or diamond, silver-plated nickel screen printed electrodes,  
10 metal oxides, metal sulfides, nanoporous titanium oxide, tin oxide coated glass, cerium  
oxide particles, molybdenum sulfide, boron nitride nanotubes, aerogels modified with  
carbon, solgels modified with carbon, ruthenium carbon aerogels and mesoporous  
silicas modified with carbon; silicon or germanium, which can be doped with  
phosphorus, boron, gallium, arsenic, indium or antimony.

15. (currently amended) The bioanode ~~biofuel cell~~ of claim 13 wherein the  
electron conductor comprises a carbon-based material.

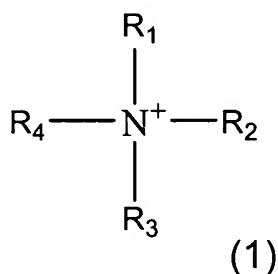
16. (currently amended) The bioanode ~~biofuel cell~~ of claim 15 wherein the  
electron conductor comprises carbon cloth, carbon paper, carbon screen printed  
electrodes, carbon black, carbon powder, carbon fiber, single-walled carbon nanotubes,  
double-walled carbon nanotubes, multi-walled carbon nanotubes, carbon nanotube  
5 arrays, diamond-coated conductors, glass carbon, mesoporous carbon, graphite,  
uncompressed graphite worms, delaminated purified flake graphite, high performance  
graphite, highly ordered pyrolytic graphite, pyrolytic graphite or polycrystalline graphite.

17. (currently amended) The bioanode ~~biofuel cell~~ of claim 6 ~~2~~ wherein the  
enzyme immobilization material is modified with a hydrophobic cation larger than  $\text{NH}_4^+$ .

18. (currently amended) The bioanode ~~biofuel cell~~ of claim 17 wherein the  
hydrophobic cation comprises an ammonium-based cation, quaternary ammonium  
cation, alkyltrimethylammonium cation, organic cation, phosphonium cation,

5 triphenylphosphonium, pyridinium cation, imidazolium cation, hexdecylpyridinium, ethidium, viologen, methyl viologen, benzyl viologen, bis(triphenylphosphine)iminium, metal complex, bipyridyl metal complex, phenanthroline-based metal complex, [Ru(bipyridine)<sub>3</sub>]<sup>2+</sup> or [Fe(phenanthroline)<sub>3</sub>]<sup>3+</sup>.

19. (currently amended) The bioanode ~~biofuel cell~~ of claim 17 wherein the hydrophobic cation comprises a quaternary ammonium cation represented by formula 1



5 wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, hydrocarbyl, substituted hydrocarbyl or heterocyclo wherein at least one of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> is other than hydrogen.

20. (currently amended) The bioanode ~~biofuel cell~~ of claim 19 wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl wherein at least one of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> is other than hydrogen.

21. (currently amended) The bioanode ~~biofuel cell~~ of claim 19 wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are the same and are methyl, ethyl, propyl, butyl, pentyl or hexyl.

22. (currently amended) The bioanode ~~biofuel cell~~ of claim 19 wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are butyl.

23. (currently amended) The bioanode ~~biofuel cell~~ of claim 6 2 wherein the enzyme comprises an oxidoreductase.

24. (currently amended) The bioanode ~~biofuel cell~~ of claim 6 2 wherein the enzyme comprises a dehydrogenase.

25. (currently amended) The bioanode ~~biofuel cell~~ of claim 6 2 wherein the enzyme comprises an alcohol dehydrogenase, aldehyde dehydrogenase, formate dehydrogenase, formaldehyde dehydrogenase, glucose dehydrogenase, glucose oxidase, lactatic dehydrogenase, lactose dehydrogenase or pyruvate dehydrogenase.

26. (currently amended) The bioanode ~~biofuel cell~~ of claim 25 wherein the enzyme comprises an alcohol dehydrogenase.

27. (currently amended) The biofuel cell of claim 114 2 wherein the bioanode and the cathode are separated by a salt bridge or a polymer electrolyte membrane.

28. (original) The biofuel cell of claim 27 wherein the bioanode and the cathode are separated by a polymer electrolyte membrane wherein the bioanode, polymer electrolyte membrane and cathode are fabricated into a membrane electrode assembly.

29. (original) The biofuel cell of claim 28 wherein the polymer electrolyte membrane comprises a perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer.

30. (currently amended) The biofuel cell of claim 114 2 further comprising a solution of a fuel fluid.

31. (original) The biofuel cell of claim 30 wherein the fuel fluid comprises ammonia, methanol, ethanol, propanol, isobutanol, butanol and isopropanol, allyl alcohols, aryl alcohols, glycerol, propanediol, mannitol, glucuronate, aldehyde, carbohydrates, glucose, glucose-1, D-glucose, L-glucose, glucose-6-phosphate, lactate, 5 lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose, sorbose, mannose, glycerate, coenzyme A, acetyl Co-A, malate, isocitrate, formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-hydroxysteroid, alpha-hydroxysteroid, lactaldehyde, testosterone, gluconate, fatty acids, lipids, phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, long-chain alcohols, 10 coniferyl-alcohol, cinnamyl-alcohol, formate, long-chain aldehydes, pyruvate, butanal, acyl-CoA, steroids, amino acids, flavin, NADH, NADH<sub>2</sub>, NADPH, NADPH<sub>2</sub> or hydrogen.

32. (original) The biofuel cell of claim 31 wherein the fuel fluid comprises methanol, ethanol or propanol.

33. (original) The biofuel cell of claim 32 wherein the fuel fluid comprises ethanol.

34. (currently amended) The biofuel cell of claim 114 2 wherein the electron mediator is in solution.

35. (currently amended) The biofuel cell of claim 114 2 wherein the cathode comprises a biocathode.

36. (currently amended) The bioanode ~~biofuel cell~~ of claim 2 wherein the electron conductor comprises an uncompressed graphite worm treated with the electrocatalyst for the electron mediator.



37. (currently amended) The bioanode biofuel cell of claim 36 wherein the electrocatalyst for the electron mediator comprises methylene green.

38. (currently amended) The bioanode biofuel cell of claim 2 wherein the electrocatalyst for the electron mediator comprises an azine, a conducting polymer or an electroactive polymer.

39. (currently amended) The bioanode biofuel cell of claim 2 wherein the electrocatalyst for the electron mediator comprises methylene green, methylene blue, luminol, nitro-fluorenone derivatives, azines, osmium phenanthroline-dione, catechol-pendant terpyridine, toluene blue, cresyl blue, nile blue, neutral red, phenazine derivatives, tionin, azure A, azure B, toluidine blue O, acetophenone, 5 metallophthalocyanines, nile blue A, modified transition metal ligands, 1,10-phenanthroline-5,6-dione, 1,10-phenanthroline-5,6-diol, [Re(phen-dione)(CO)<sub>3</sub>Cl], [Re(phen-dione)<sub>3</sub>](PF<sub>6</sub>)<sub>2</sub>, poly(metallophthalocyanine), poly(thionine), quinones, diimines, diaminobenzenes, diaminopyridines, phenothiazine, phenoxazine, toluidine 10 blue, brilliant cresyl blue, 3,4-dihydroxybenzaldehyde, poly(acrylic acid), poly(azure I), poly(nile blue A), poly(methylene green), poly(methylene blue), polyaniline, polypyridine, polypyrrole, polythiophene, poly(thieno[3,4-*b*]thiophene), poly(3-hexylthiophene), poly(3,4-ethylenedioxythiophene), poly(isothianaphthene), poly(3,4-ethylenedioxythiophene), poly(difluoroacetylene), poly(4-dicyanomethylene-4H- 15 cyclopenta[2,1-*b*;3,4-*b'*]dithiophene), poly(3-(4-fluorophenyl)thiophene) or poly(neutral red).

40. (currently amended) The bioanode biofuel cell of claim 2 wherein the electrocatalyst for the electron mediator comprises methylene green.

41. (currently amended) The bioanode biofuel cell of claim 2 wherein the electrocatalyst for the electron mediator comprises poly(methylene green).

42. (currently amended) The bioanode biofuel cell of claim 6 2 wherein the enzyme immobilization material comprises perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer, modified perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer, polysulfone, micellar polymers, poly(ethylene oxide) based block  
5 copolymers, polymers formed from microemulsion, polymers formed from micellar polymerization, copolymers of alkyl methacrylates, alkyl acrylates and styrenes, ceramics, sodium bis(2-ethylhexyl)sulfosuccinate, sodium dioctylsulfonsuccinate, lipids, phospholipids, sodium dodecyl sulfate, decyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, (4-[(2-hydroxyl-1-  
10 naphthalenyl)azo]benzenesulfonic acid monosodium salt), linoleic acids, linolenic acids, colloids, liposomes or micelle networks.

43. (currently amended) The bioanode biofuel cell of claim 42 wherein the enzyme immobilization material comprises a perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer.

44. (currently amended) The bioanode biofuel cell of claim 42 wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer.

45. (currently amended) The bioanode biofuel cell of claim 2 wherein the electron mediator comprises nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) or nicotinamide adenine dinucleotide phosphate (NADP).

46. (currently amended) The bioanode biofuel cell of claim 6 2 wherein the electron mediator comprises pyrroloquinoline quinone, phenazine methosulfate, dichlorophenol indophenol, short chain ubiquinones or potassium ferricyanide.

47. (currently amended) The bioanode biofuel cell of claim 12 wherein the electron conductor comprises an uncompressed graphite worm treated with poly(methylene green), the modified perfluoro sulfonic acid-PTFE copolymer is modified with a tetrabutylammonium ion, the enzyme comprises an alcohol dehydrogenase and further comprises a solution containing ethanol and  $\text{NAD}^+$ .

48. (currently amended) A method of generating electricity using the biofuel cell of claim 113 2 comprising

- (a) oxidizing the fuel fluid at the bioanode and reducing the oxidant at the cathode;
- (b) reducing the oxidized form of the electron mediator during the oxidization of the fuel fluid at the bioanode;
- (c) reducing the electrocatalyst; and
- (d) oxidizing the electrocatalyst at the electron conductor.

49. (currently amended) A method of generating electricity using the biofuel cell of claim 114 6 comprising

- (a) oxidizing the fuel fluid at the bioanode and reducing the oxidant at the cathode;
- (b) reducing the oxidized form of the electron mediator during the oxidization of the fuel fluid at the bioanode; and
- (c) oxidizing the electron mediator at the electron conductor.

50. (currently amended) The method of claim 49 48 wherein the fuel fluid comprises ammonia, methanol, ethanol, propanol, isobutanol, butanol and isopropanol, allyl alcohols, aryl alcohols, glycerol, propanediol, mannitol, glucuronate, aldehyde,

carbohydrates, glucose, glucose-1, D-glucose, L-glucose, glucose-6-phosphate, lactate,  
5 lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose,  
sorbose, mannose, glycerate, coenzyme A, acetyl Co-A, malate, isocitrate,  
formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-hydroxysteroid, alpha-  
hydroxysteroid, lactaldehyde, testosterone, gluconate, fatty acids, lipids,  
phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, long-chain alcohols,  
10 coniferyl-alcohol, cinnamyl-alcohol, formate, long-chain aldehydes, pyruvate, butanal,  
acyl-CoA, steroids, amino acids, flavin, NADH, NADH<sub>2</sub>, NADPH, NADPH<sub>2</sub> or hydrogen.

51. (currently amended) The method of claim 49 48 wherein the fuel fluid  
comprises methanol, ethanol or propanol.

52. (original) The method of claim 50 wherein the fuel fluid comprises ethanol.

53. (original) The method of claim 48 wherein the electrocatalyst for an electron  
mediator comprises an azine, a conducting polymer or an electroactive polymer.

54. (original) The method of claim 48 wherein the electrocatalyst for the electron  
mediator comprises methylene green, methylene blue, luminol, nitro-fluorenone  
derivatives, azines, osmium phenanthroline-dione, catechol-pendant terpyridine, toluene  
blue, cresyl blue, nile blue, neutral red, phenazine derivatives, tironin, azure A, azure B,  
5 toluidine blue O, acetophenone, metallophthalocyanines, nile blue A, modified transition  
metal ligands, 1,10-phenanthroline-5,6-dione, 1,10-phenanthroline-5,6-diol, [Re(phen-  
dione)(CO)<sub>3</sub>Cl], [Re(phen-dione)<sub>3</sub>](PF<sub>6</sub>)<sub>2</sub>, poly(metallophthalocyanine), poly(thionine),  
quinones, diimines, diaminobenzenes, diaminopyridines, phenothiazine, phenoxazine,  
toluidine blue, brilliant cresyl blue, 3,4-dihydroxybenzaldehyde, poly(acrylic acid),  
10 poly(azure I), poly(nile blue A), poly(methylene green), poly(methylene blue),  
polyaniline, polypyridine, polypyrrole, polythiophene, poly(thieno[3,4-*b*]thiophene),

poly(3-hexylthiophene), poly(3,4-ethylenedioxythiophene), poly(isothianaphthene),  
poly(3,4-ethylenedioxythiophene), poly(difluoroacetylene), poly(4-dicyanomethylene-4H-  
cyclopenta[2,1-*b*;3,4-*b'*]dithiophene), poly(3-(4-fluorophenyl)thiophene) or poly(neutral  
red).

55. (original) The method of claim 54 wherein the electrocatalyst for the electron mediator comprises methylene green.

56. (original) The method of claim 54 wherein the electrocatalyst for the electron mediator comprises poly(methylene green).

57. (original) The method of claim 48 wherein the electron mediator comprises nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) or nicotinamide adenine dinucleotide phosphate (NADP).

58. (original) The method of claim 57 wherein the electron mediator comprises  $\text{NAD}^+$ .

59. (original) The method of claim 48 wherein the electron conductor comprises an uncompressed graphite worm treated with poly(methylene green), the modified perfluoro sulfonic acid-PTFE copolymer is modified with a tetrabutylammonium ion, the enzyme comprises an alcohol dehydrogenase, the fuel fluid comprises ethanol and the  
5 electron mediator comprises  $\text{NAD}^+$ .

60. (original) The method of claim 49 wherein the electron mediator comprises pyrroloquinoline quinone, phenazine methosulfate, dichlorophenol indophenol, short chain ubiquinones or potassium ferricyanide.

61. (original) The method of claim 60 wherein the electron mediator comprises pyrroloquinoline quinone (PQQ).

62. (original) The method of claim 49 wherein the electron conductor comprises carbon cloth, the modified perfluoro sulfonic acid-PTFE copolymer is modified with a tetrabutylammonium ion, the enzyme comprises an alcohol dehydrogenase, the fuel fluid comprises ethanol and the electron mediator comprises PQQ.

63. (original) An enzyme immobilized in a non-naturally occurring colloidal immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to a compound smaller than the enzyme.

64. (original) An enzyme immobilized in an acellular, colloidal immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to a compound smaller than the enzyme.

65. (original) An enzyme immobilized in a micellar or inverted micellar immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to a compound smaller than the enzyme.

66. (original) An enzyme immobilized in a cation-modified perfluoro sulfonic acid-PTFE copolymer capable of immobilizing and stabilizing the enzyme, the material being permeable to a compound smaller than the enzyme.

67. (original) The immobilized enzyme of claim 65 wherein the enzyme comprises an alcohol dehydrogenase, aldehyde dehydrogenase, formate dehydrogenase, formaldehyde dehydrogenase, glucose dehydrogenase, glucose oxidase, lactic dehydrogenase, lactose dehydrogenase or pyruvate dehydrogenase.

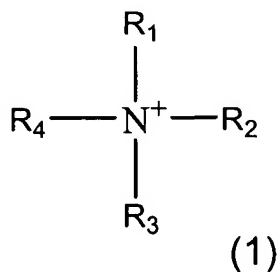
68. (original) The immobilized enzyme of claim 65 wherein the enzyme is active for at least about 30, 60, 90, 120, 150, 180, 240, 300 or 365 days.

5 69. (original) The immobilized enzyme of claim 65 wherein the immobilization material comprises a cation modified perfluoro sulfonic acid-PTFE copolymer.

70. (original) The immobilized enzyme of claim 69 wherein the cation modified perfluoro sulfonic acid-PTFE copolymer is modified with a hydrophobic cation larger  
10 than  $\text{NH}_4^+$ .

71. (original) The immobilized enzyme of claim 70 wherein the hydrophobic cation comprises an ammonium-based cation, quaternary ammonium cation, alkyltrimethylammonium cation, organic cation, phosphonium cation, triphenylphosphonium, pyridinium cation, imidazolium cation, hexdecylpyridinium,  
5 ethidium, viologen, methyl viologen and benzyl viologen, bis(triphenylphosphine)iminium, metal complex, bipyridyl metal complex, phenanthroline-based metal complex,  $[\text{Ru}(\text{bipyridine})_3]^{2+}$  or  $[\text{Fe}(\text{phenanthroline})_3]^{3+}$ .

72. (original) The immobilized enzyme of claim 70 wherein the hydrophobic cation comprises a quaternary ammonium cation represented by formula 1



wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently hydrogen, hydrocarbyl, substituted hydrocarbyl or heterocyclo wherein at least one of  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  is other than hydrogen.

73. (original) The immobilized enzyme of claim 72 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl wherein at least one of  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  is other than hydrogen.

74. (original) The immobilized enzyme of claim 72 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are the same and are methyl, ethyl, propyl, butyl, pentyl or hexyl.

75. (original) The immobilized enzyme of claim 72 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are butyl.

76. (original) The immobilized enzyme of claim 65 wherein the compounds smaller than the enzymes comprise ammonia, methanol, ethanol, propanol, isobutanol, butanol and isopropanol, allyl alcohols, aryl alcohols, glycerol, propanediol, mannitol, glucuronate, aldehyde, carbohydrates, glucose, glucose-1, D-glucose, L-glucose, 5 glucose-6-phosphate, lactate, lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose, sorbose, mannose, glycerate, coenzyme A, acetyl Co-A, malate, isocitrate, formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-hydroxysteroid, alpha-hydroxysteroid, lactaldehyde, testosterone, gluconate, fatty acids, lipids, phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, long-chain 10 alcohols, coniferyl-alcohol, cinnamyl-alcohol, formate, long-chain aldehydes, pyruvate, butanal, acyl-CoA, steroids, amino acids, flavin, NADH, NADH<sub>2</sub>, NADPH, NADPH<sub>2</sub> or hydrogen.



77. (original) Use of the immobilized enzyme of claim 65 in a biofuel cell, a biosensor, a bioprocessor, a bioassay, an enzyme sensor, a bioreactor, enzyme therapy, an immunoassay or a biomimic.

Claims 78. – 112. (cancelled)

113. (new) A biofuel cell for generating electricity comprising:  
a fuel fluid;  
a cathode capable of reducing an oxidant in the presence of electrons to form  
water; and  
5 a bioanode of claim 2.

114. (new) A biofuel cell for generating electricity comprising:  
a fuel fluid;  
a cathode capable of reducing an oxidant in the presence of electrons to form  
water; and  
5 a bioanode of claim 6.

115. (new) A biofuel cell for generating electricity comprising:  
a fuel fluid;  
an electron mediator;  
a cathode capable of reducing an oxidant in the presence of electrons to form  
5 water; and  
a bioanode of claim 1.

116. (new) A biofuel cell for generating electricity comprising:  
a fuel fluid;  
an electron mediator;

a cathode capable of reducing an oxidant in the presence of electrons to form  
5 water; and  
a bioanode of claim 5.

117. (new) The biofuel cell of claim 114 wherein the electron conductor  
comprises a carbon-based material, a metallic conductor, a semiconductor, a metal  
oxide or a modified conductor.

118. (new) The biofuel cell of claim 117 wherein the electron conductor  
comprises carbon cloth, carbon paper, carbon screen printed electrodes, carbon black,  
carbon powder, carbon fiber, single-walled carbon nanotubes, double-walled carbon  
nanotubes, multi-walled carbon nanotubes, carbon nanotube arrays, diamond-coated  
5 conductors, glass carbon, mesoporous carbon, graphite, uncompressed graphite  
worms, delaminated purified flake graphite, high performance graphite, highly ordered  
pyrolytic graphite, polycrystalline graphite, gold, platinum, iron, nickel,  
copper, silver, stainless steel, mercury, tungsten, nanoparticles made of cobalt or  
diamond, silver-plated nickel screen printed electrodes, metal oxides, metal sulfides,  
10 nanoporous titanium oxide, tin oxide coated glass, cerium oxide particles, molybdenum  
sulfide, boron nitride nanotubes, aerogels modified with carbon, solgels modified with  
carbon, ruthenium carbon aerogels and mesoporous silicas modified with carbon;  
silicon or germanium, which can be doped with phosphorus, boron, gallium, arsenic,  
indium or antimony.

119. (new) The biofuel cell of claim 118 wherein the electron conductor  
comprises a carbon-based material.

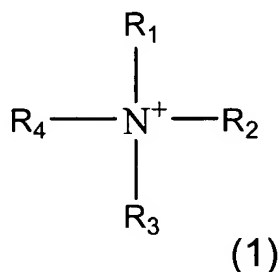
120. (new) The biofuel cell of claim 119 wherein the electron conductor  
comprises carbon cloth, carbon paper, carbon screen printed electrodes, carbon black,

carbon powder, carbon fiber, single-walled carbon nanotubes, double-walled carbon nanotubes, multi-walled carbon nanotubes, carbon nanotube arrays, diamond-coated  
5 conductors, glass carbon, mesoporous carbon, graphite, uncompressed graphite worms, delaminated purified flake graphite, high performance graphite, highly ordered pyrolytic graphite, pyrolytic graphite or polycrystalline graphite.

121. (new) The biofuel cell of claim 114 wherein the enzyme immobilization material is modified with a hydrophobic cation larger than  $\text{NH}_4^+$ .

122. (new) The biofuel cell of claim 121 wherein the hydrophobic cation comprises an ammonium-based cation, quaternary ammonium cation, alkyltrimethylammonium cation, organic cation, phosphonium cation, triphenylphosphonium, pyridinium cation, imidazolium cation, hexdecylpyridinium,  
5 ethidium, viologen, methyl viologen, benzyl viologen, bis(triphenylphosphine)iminium, metal complex, bipyridyl metal complex, phenanthroline-based metal complex,  $[\text{Ru}(\text{bipyridine})_3]^{2+}$  or  $[\text{Fe}(\text{phenanthroline})_3]^{3+}$ .

123. (new) The biofuel cell of claim 121 wherein the hydrophobic cation comprises a quaternary ammonium cation represented by formula 1



5 wherein  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$  and  $\text{R}_4$  are independently hydrogen, hydrocarbyl, substituted hydrocarbyl or heterocyclo wherein at least one of  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$  and  $\text{R}_4$  is other than hydrogen.

124. (new) The biofuel cell of claim 123 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl wherein at least one of  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  is other than hydrogen.

125. (new) The biofuel cell of claim 123 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are the same and are methyl, ethyl, propyl, butyl, pentyl or hexyl.

126. (new) The biofuel cell of claim 123 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are butyl.

127. (new) The biofuel cell of claim 114 wherein the enzyme comprises an oxidoreductase.

128. (new) The biofuel cell of claim 114 wherein the enzyme comprises a dehydrogenase.

129. (new) The biofuel cell of claim 114 wherein the enzyme comprises an alcohol dehydrogenase, aldehyde dehydrogenase, formate dehydrogenase, formaldehyde dehydrogenase, glucose dehydrogenase, glucose oxidase, lactatic dehydrogenase, lactose dehydrogenase or pyruvate dehydrogenase.

130. (new) The biofuel cell of claim 129 wherein the enzyme comprises an alcohol dehydrogenase.

131. (new) A salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer having pores of a size sufficient to constrain an enzyme therein.

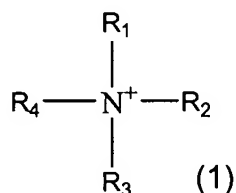
132. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 131 wherein said pores are substantially the same size and same shape as said enzyme.

133. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 131 wherein said enzyme retains at least about 75% of its initial catalytic activity for at least 365 days.

134. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 131 wherein said enzyme retains at least about 75% of its initial catalytic activity for more than about 365 days.

135. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 131 wherein said tetraalkyl-ammonium ion comprises an alkyltrimethyl ammonium cation or alkyltriethylammonium cation.

136. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 131 wherein said tetraalkyl-ammonium ion is represented by formula 1



5 wherein

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;

wherein at least one of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> is other than hydrogen.

137. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 136 wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are methyl, ethyl, propyl, butyl, pentyl or hexyl.

138. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 136 wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are butyl.

139. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 137 wherein said enzyme retains at least about 75% of its initial catalytic activity for more than about 365 days.

140. (new) The salt-extracted tetraalkyl-ammonium modified perfluoro sulfonic acid-PTFE copolymer of claim 138 wherein said enzyme retains at least about 75% of its initial catalytic activity for more than about 365 days.